



The Potential Role, Mechanisms and Relevance of Pollutant-Induced Oxidative Stress in Preterm Birth

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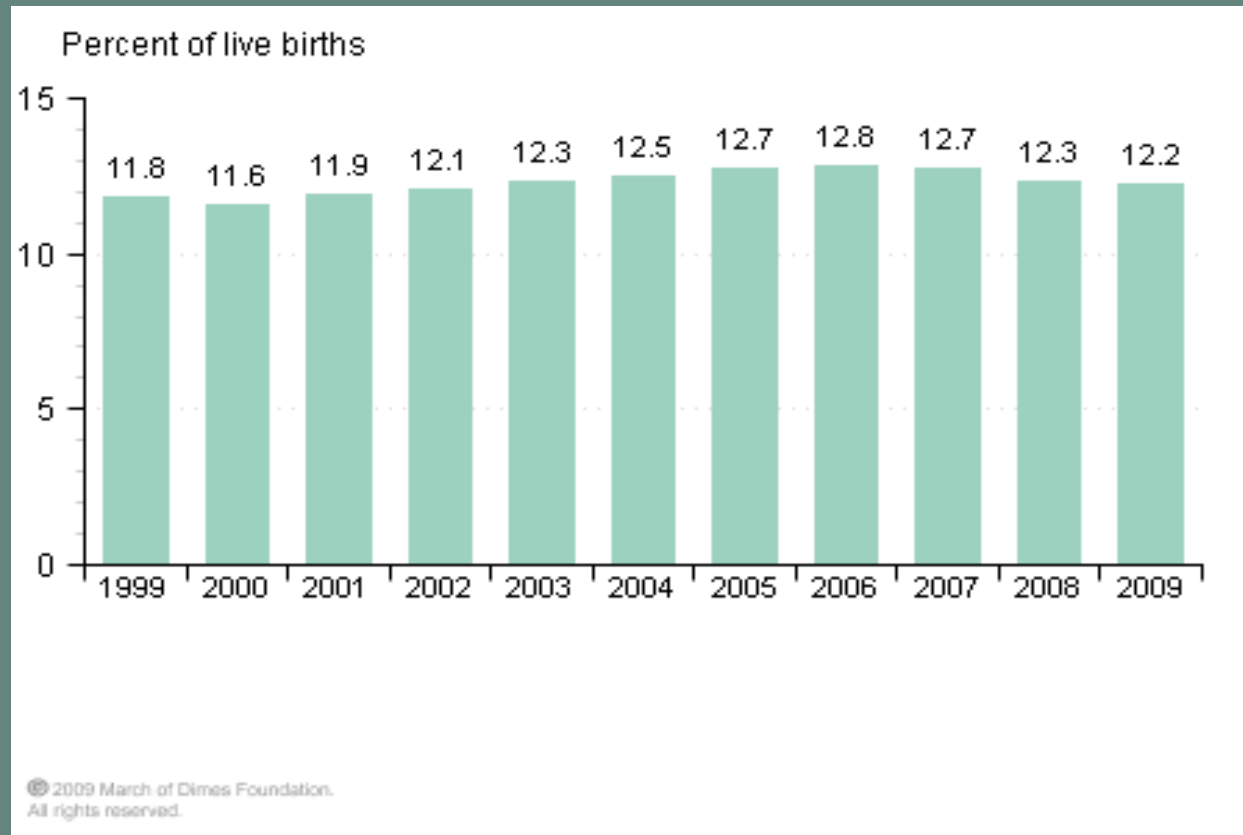
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Preterm birth

United States, 1999-2009



Preterm is less than 37 completed weeks gestation.

Source: National Center for Health Statistics, final natality data. Retrieved August 2, 2012, from www.marchofdimes.com/peristats.

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Preterm Birth Medical Consequences for Infant

- Leading factor in infant death
 - 19.7% of infant deaths
- Medical complications
 - Respiratory distress syndrome
 - Bleeding in the brain (intraventricular hemorrhage)
 - Nearly half of all congenital neurological defects (e.g., cerebral palsy)
 - Chronic lung disease (bronchopulmonary dysplasia)
 - Apnea
 - Retinopathy of prematurity (can lead to blindness)
 - Anemia
 - Infections



Cost of Preterm Birth

United States, 2005

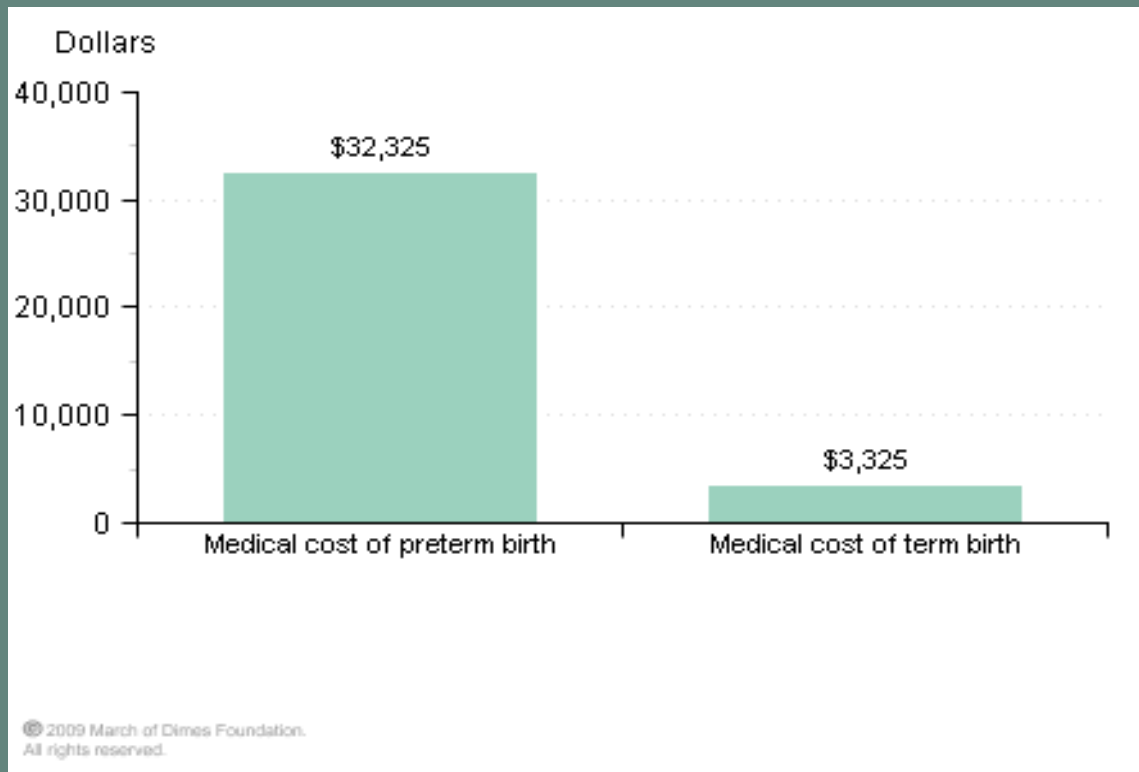


Photo by Tina Stallard. 2005 Visions of Science winner.

Preterm is less than 37 completed weeks gestation.

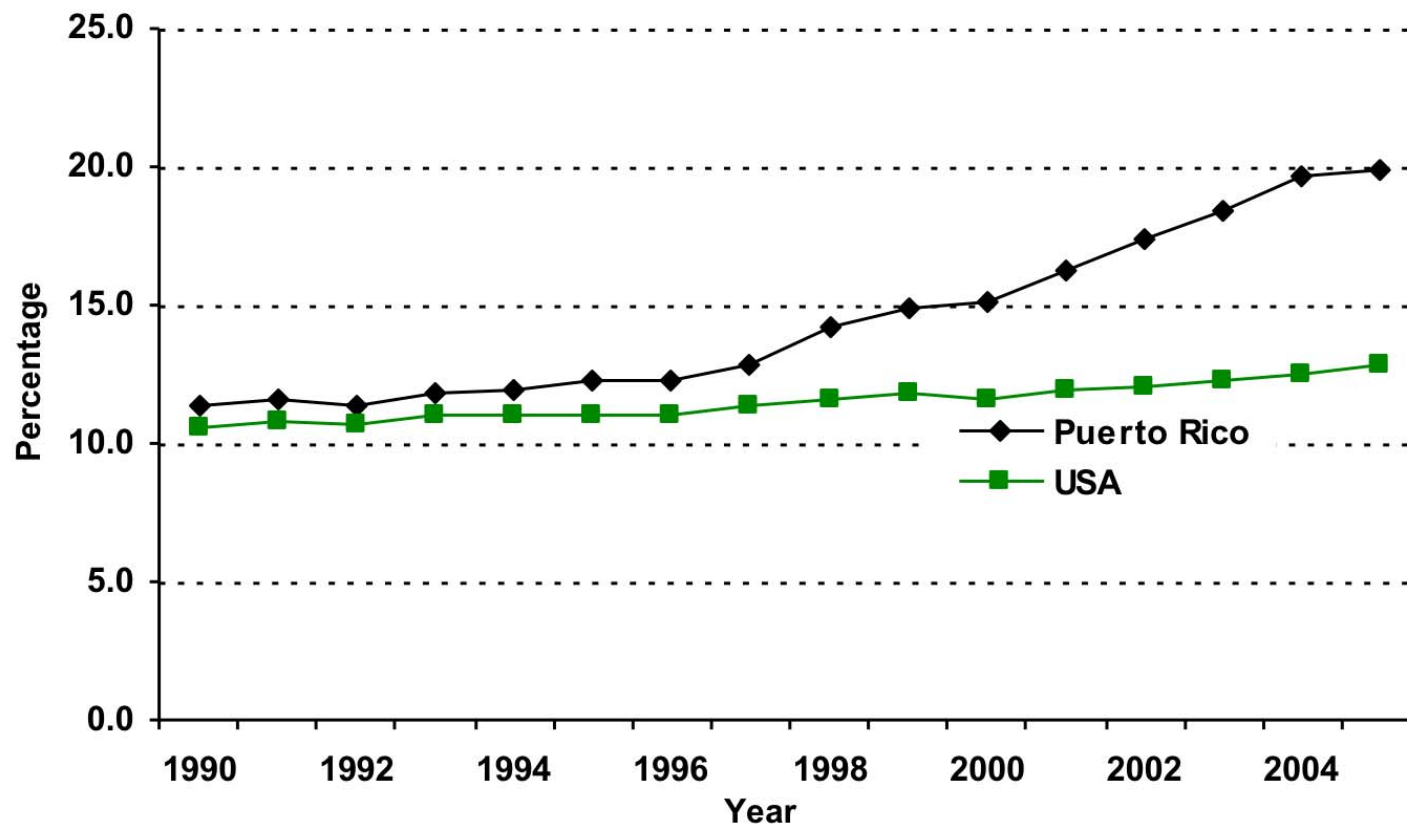
Source: Institute of Medicine. 2007. Preterm Birth: Causes, Consequences, and Prevention. National Academy Press, Washington, D.C. Published and unpublished analyses. Retrieved August 2, 2012, from www.marchofdimes.com/peristats.

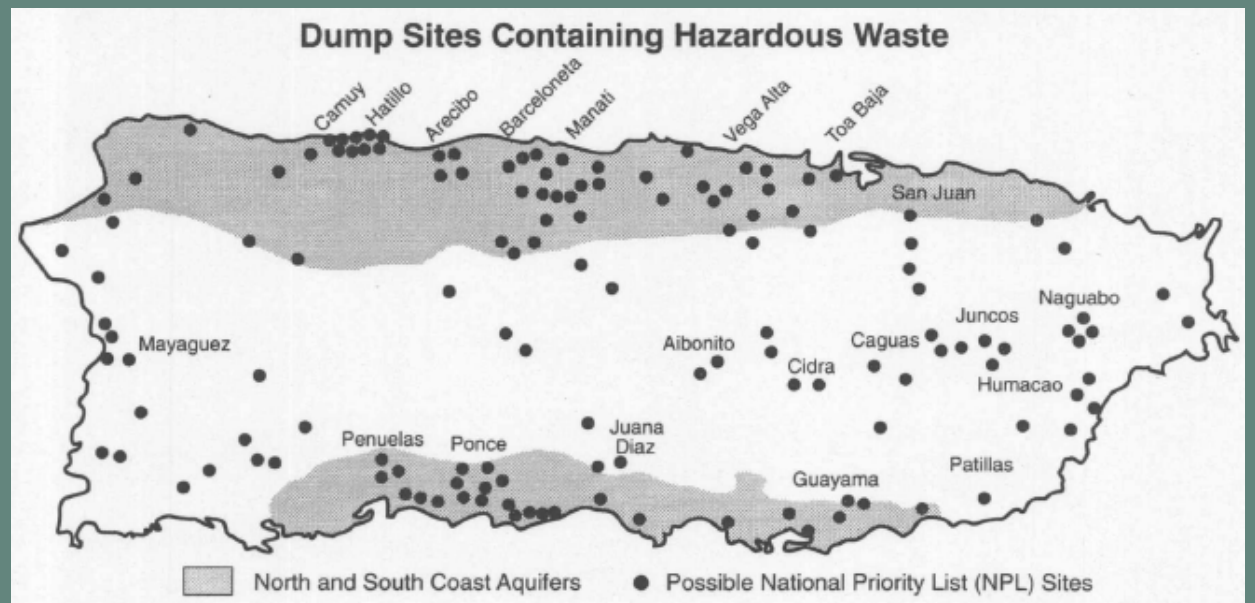
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Preterm Birth Rates in US & Puerto Rico

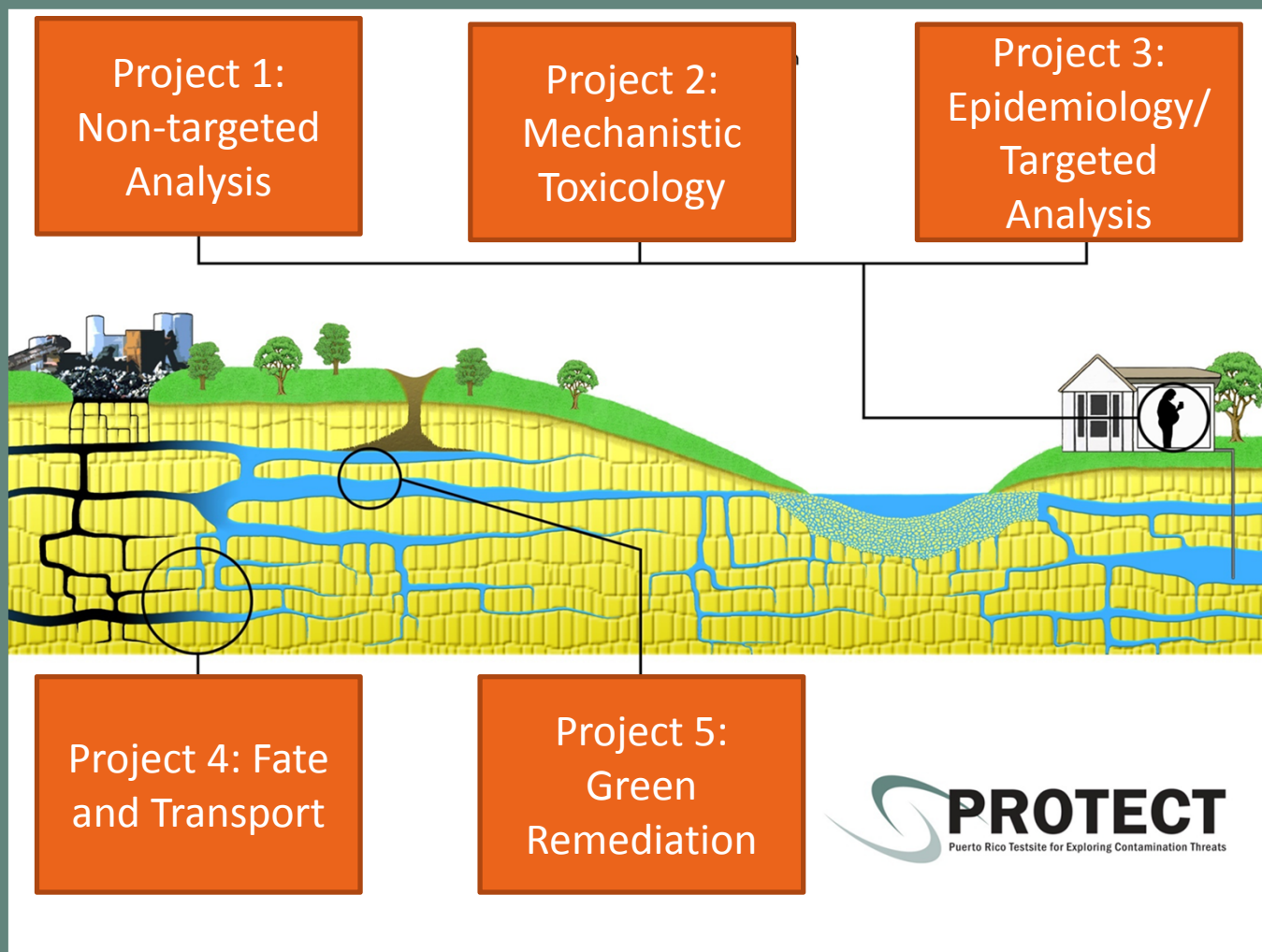
1990 - 2005





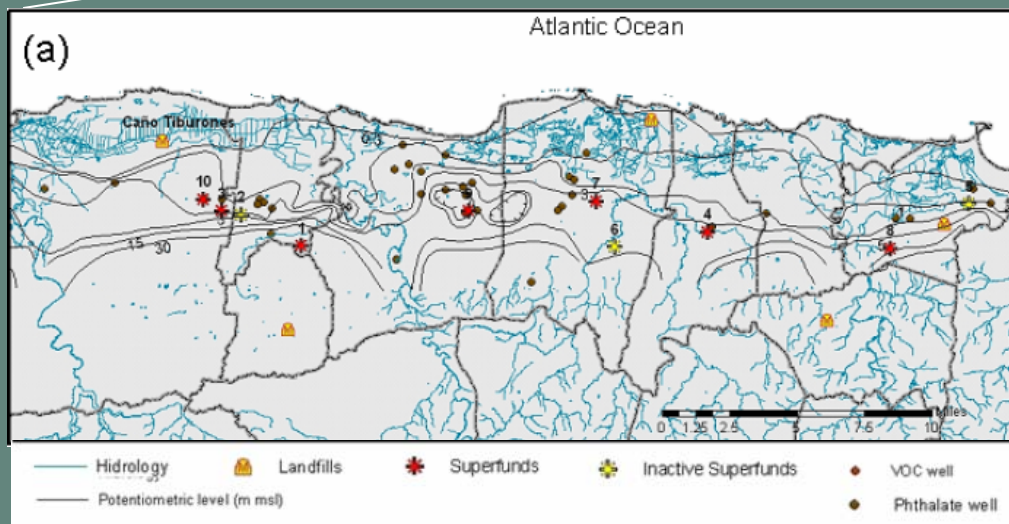
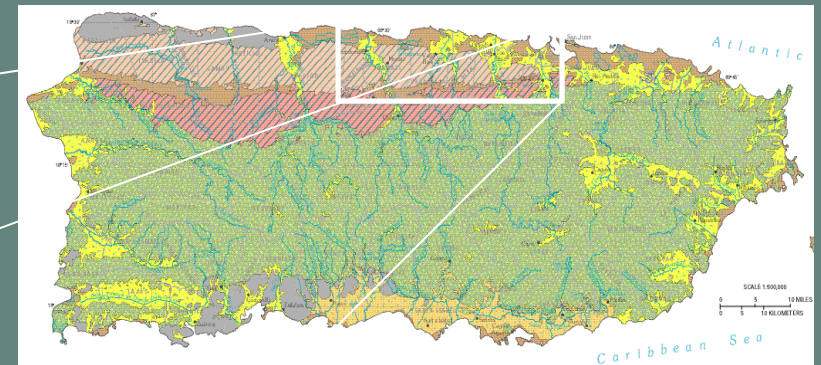


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Phthalate Contamination in Puerto Rico



Phthalates

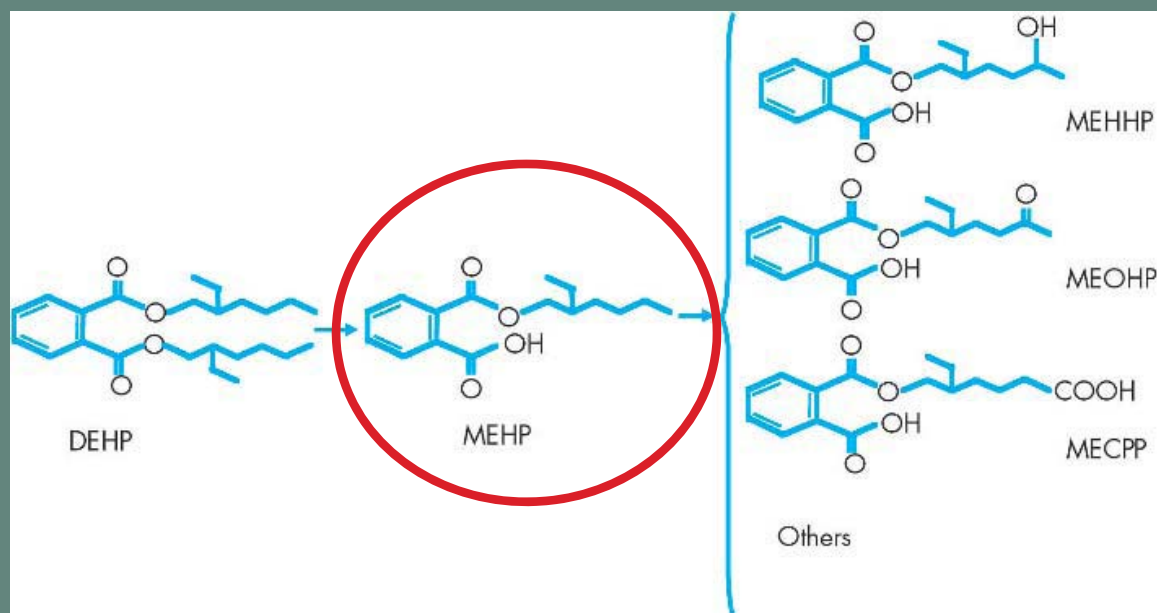
- [illegible]





Mono-2-ethylhexyl phthalate (MEHP)

Mono-2-ethylhexyl phthalate (MEHP) is a biologically active metabolite of the plasticizer di-2-ethylhexyl phthalate (DEHP)





Associations between Human Exposure to Phthalates & Birth Outcomes

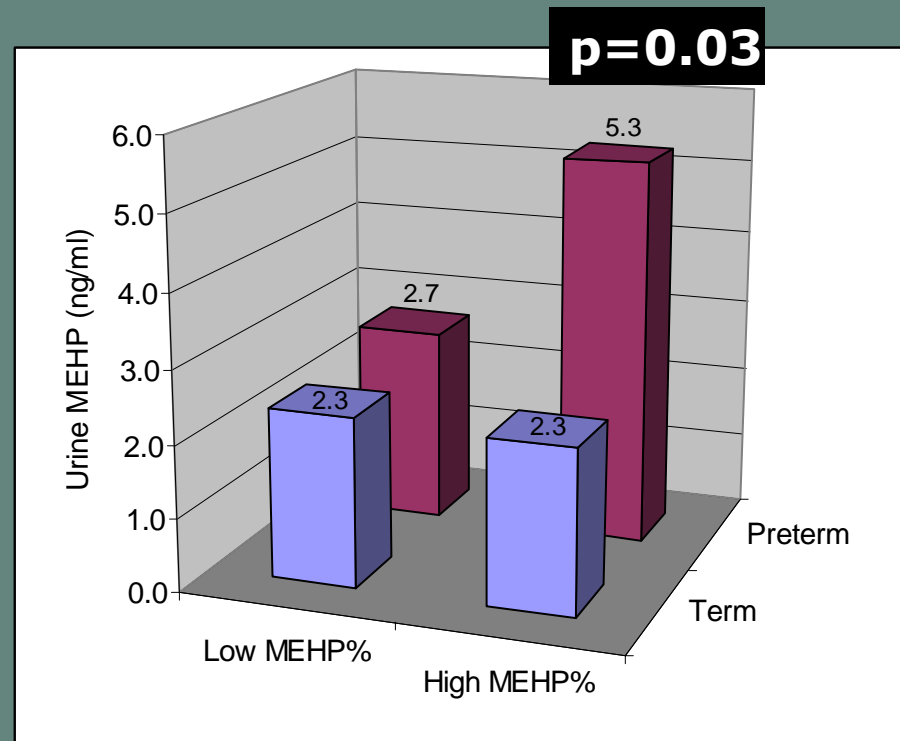
- Latini et al. 2003, *EHP* (n = 84)
 - The DEHP metabolite MEHP in cord blood was inversely and significantly associated with decreased gestation length
- Whyatt et al. 2009, *Pediatrics* (n = 311)
 - Urinary DEHP metabolites inversely associated with gestation length
 - Women with the highest MEHP quartile delivered 5 days earlier than lowest quartile
- Zhang et al. 2009, *J Pediatr* (n = 88 cases, 113 controls)
 - DEHP and DBP or metabolites in cord blood/meconium was associated with dose-dependent increased odds for low birth weight
- Wolff et al. 2008, *EHP* (n = 404)
 - Urinary MEHP associated with increased gestation length
- Adibi et al. 2009, *Am J Epidemiol* (n = 284)
 - DEHP metabolites associated with increased gestation length

Meeker JD, Ferguson KK. Phthalates: Human Exposure and Related Health Effects. In: Schecter A, Gasiewicz T, eds. Dioxins and Persistent Organic Pollutants: Health and Toxicity, 3rd Edition. John Wiley & Sons, Hoboken, NJ; In press.



DEHP Metabolite Ratios and Preterm Birth

- MEHP% is the sum of DEHP/MEHP oxidative metabolites divided by MEHP
- Geometric mean MEHP urine concentrations in women delivering term or preterm stratified by MEHP% median

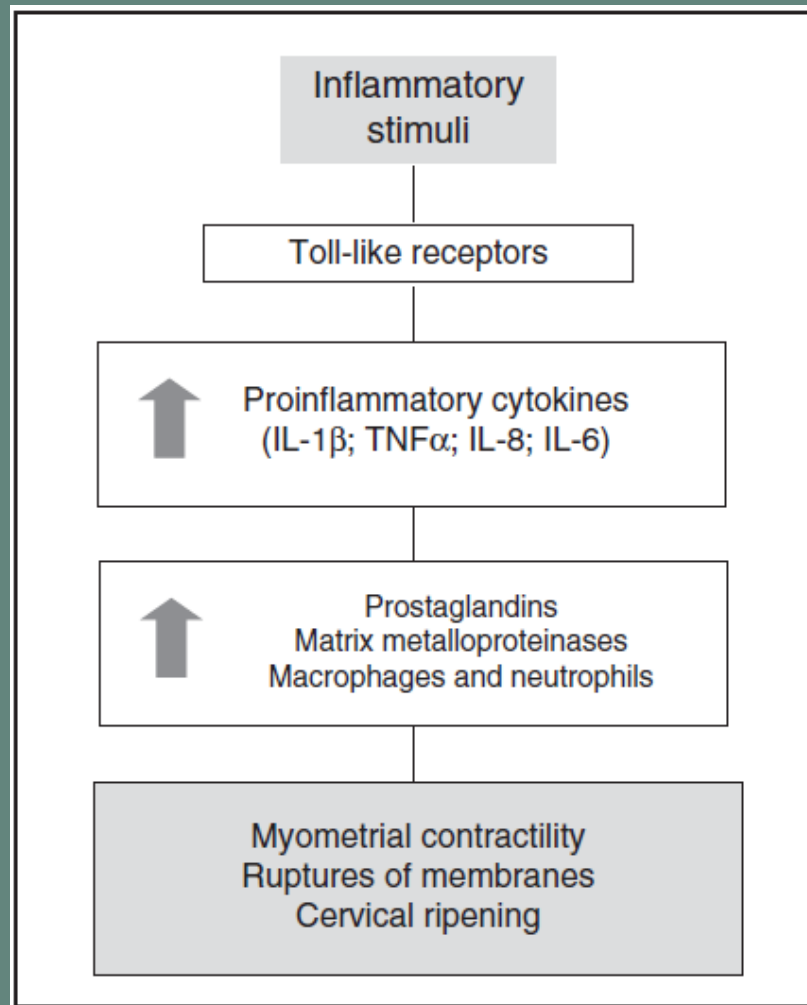


Meeker JD, et al. Urinary phthalate metabolites in relation to preterm birth in Mexico City. *Environ Health Perspect*, 117:1587-2, 2009.





Popular Model of Parturition Activation

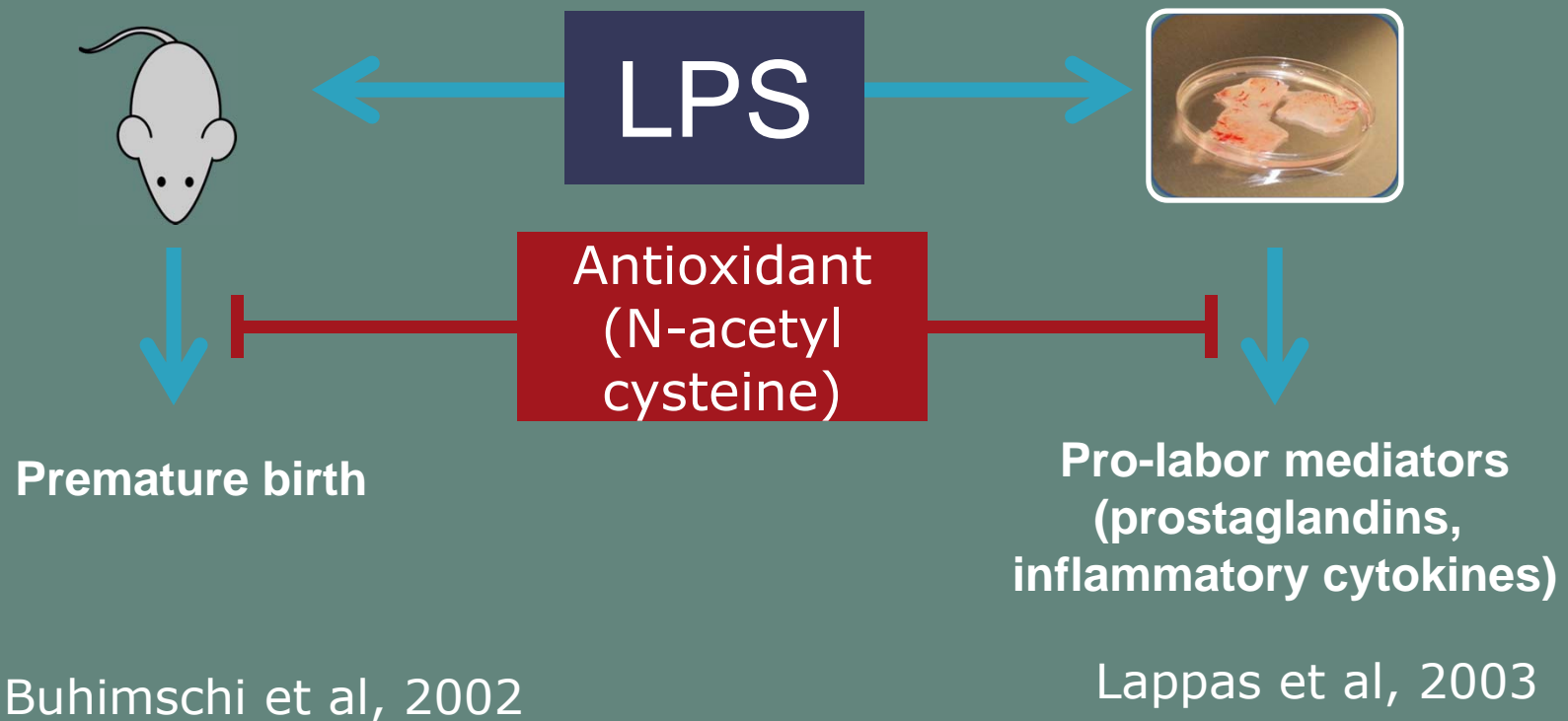


Challis, JR et al., Reprod Sci 16, 206-215, 2009





Role of Reactive Oxygen Species (ROS) in Parturition





Toxicant Activation of Parturition

Toxicants



Bacteria

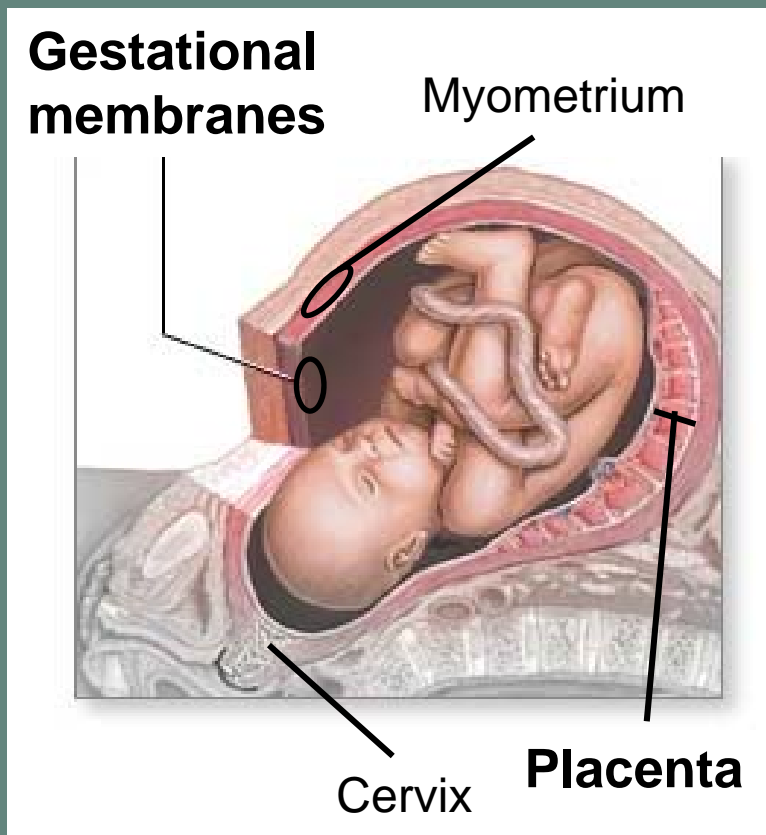


Diagram adapted from A.D.A.M Interactive Anatomy 2009.

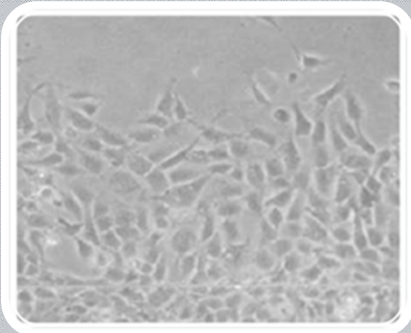
Toxicants



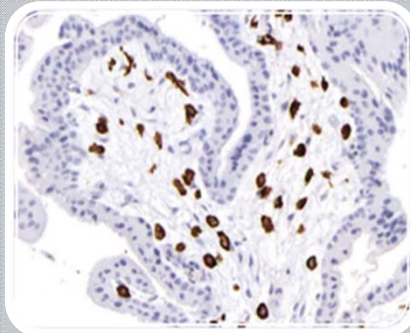
Oxidative Stress



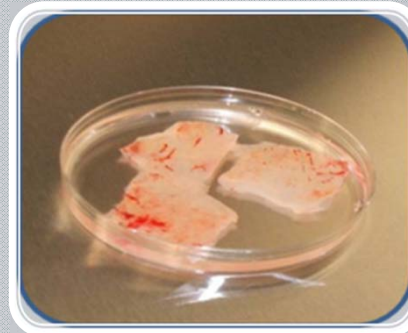
In Vitro Models



HTR-8/Svneo
cells



Primary
placental
macrophages



Gestational
membrane
tissue
explants

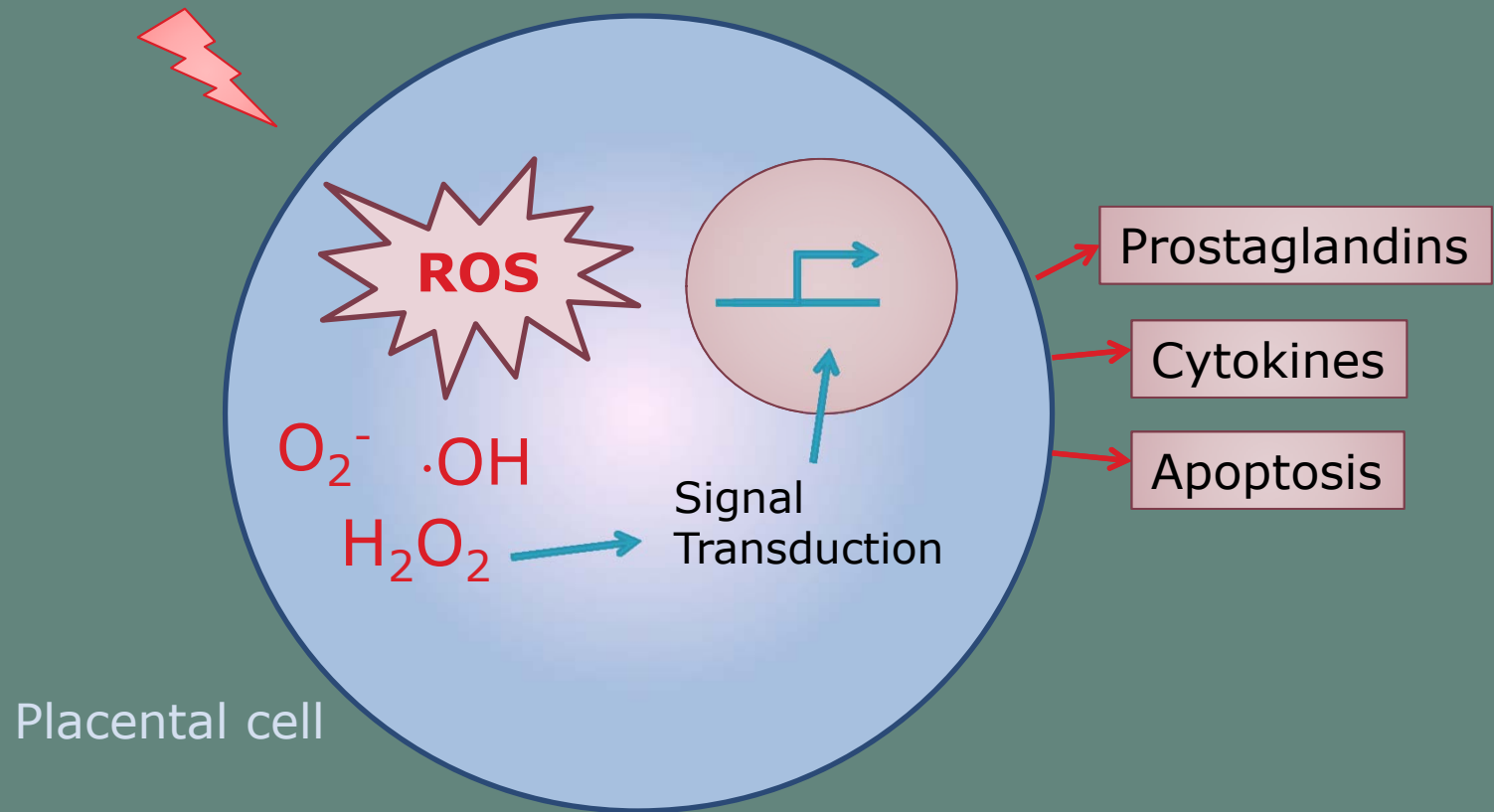


Gestational
membranes
mounted on
Transwell
inserts



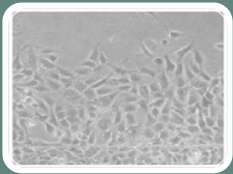
Hypothesis

tert-Butylhydroperoxide (TBHP) – Model pro-oxidant
Mono-2-ethylhexyl phthalate (MEHP) – Phthalate metabolite
S-(1,2)-Dichlorovinyl-L-cysteine (DCVC) – Trichloroethylene metabolite






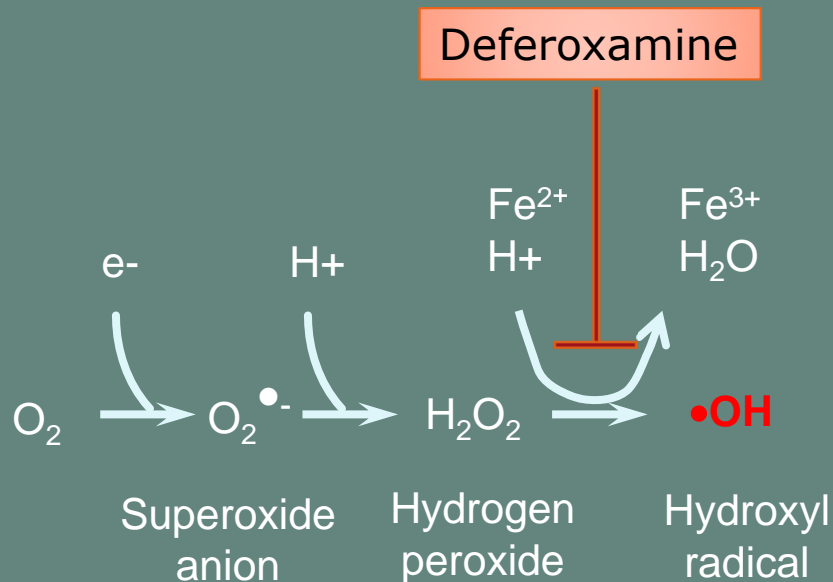
A 1-h exposure to MEHP stimulated cellular production of reactive species in HTR-8 cells as measured by dichlorofluorescein (DCF) assay



Manuscript under review



Deferoxamine Inhibits MEHP-Stimulated Generation of ROS in HTR-8 cells as measured by dichlorofluorescein (DCF) fluorescence

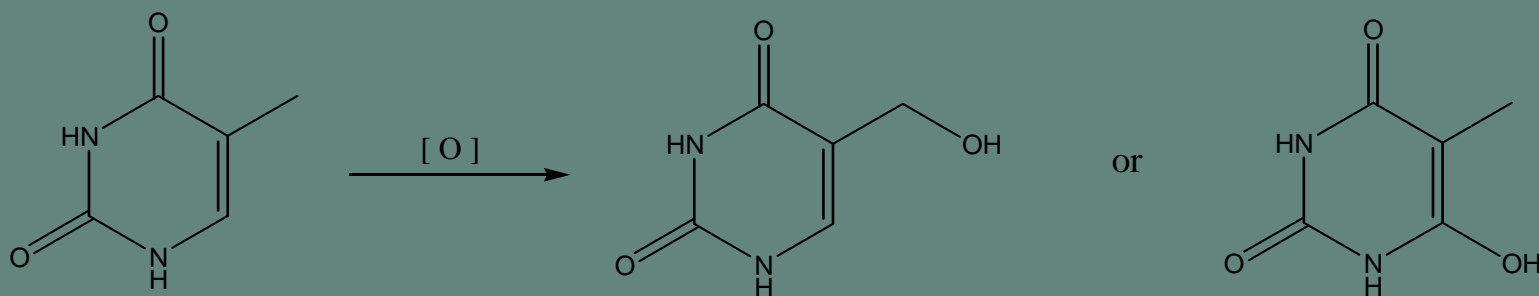


Manuscript under review



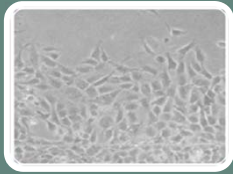
Oxidative DNA Adducts

Mass tag profiling allows improved detection of DNA adducts from oxidative stress



Thymine

Hydroxy thymine

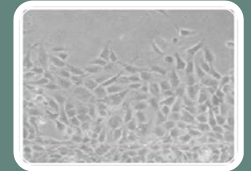


A 24-h exposure to 90 or 180 μ M MEHP increased oxidative damage to DNA of HTR-8 cells as measured by mass tag profiling for detection of hydroxylated thymine

Manuscript under review

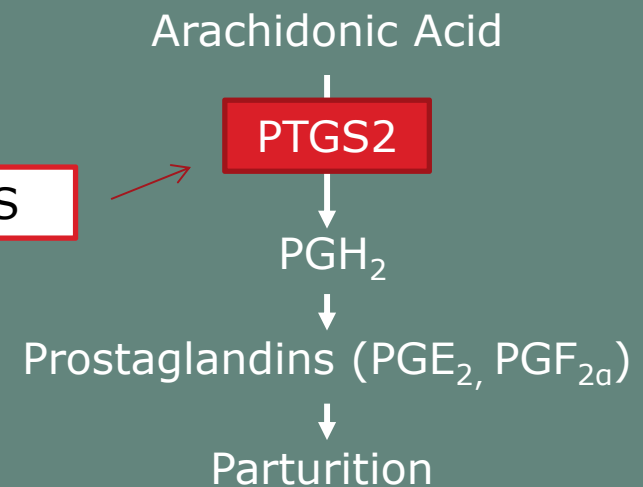


MEHP stimulated PTGS2 mRNA Expression in HTR-8 cells after 4 and 8 h of exposure



Manuscript under review

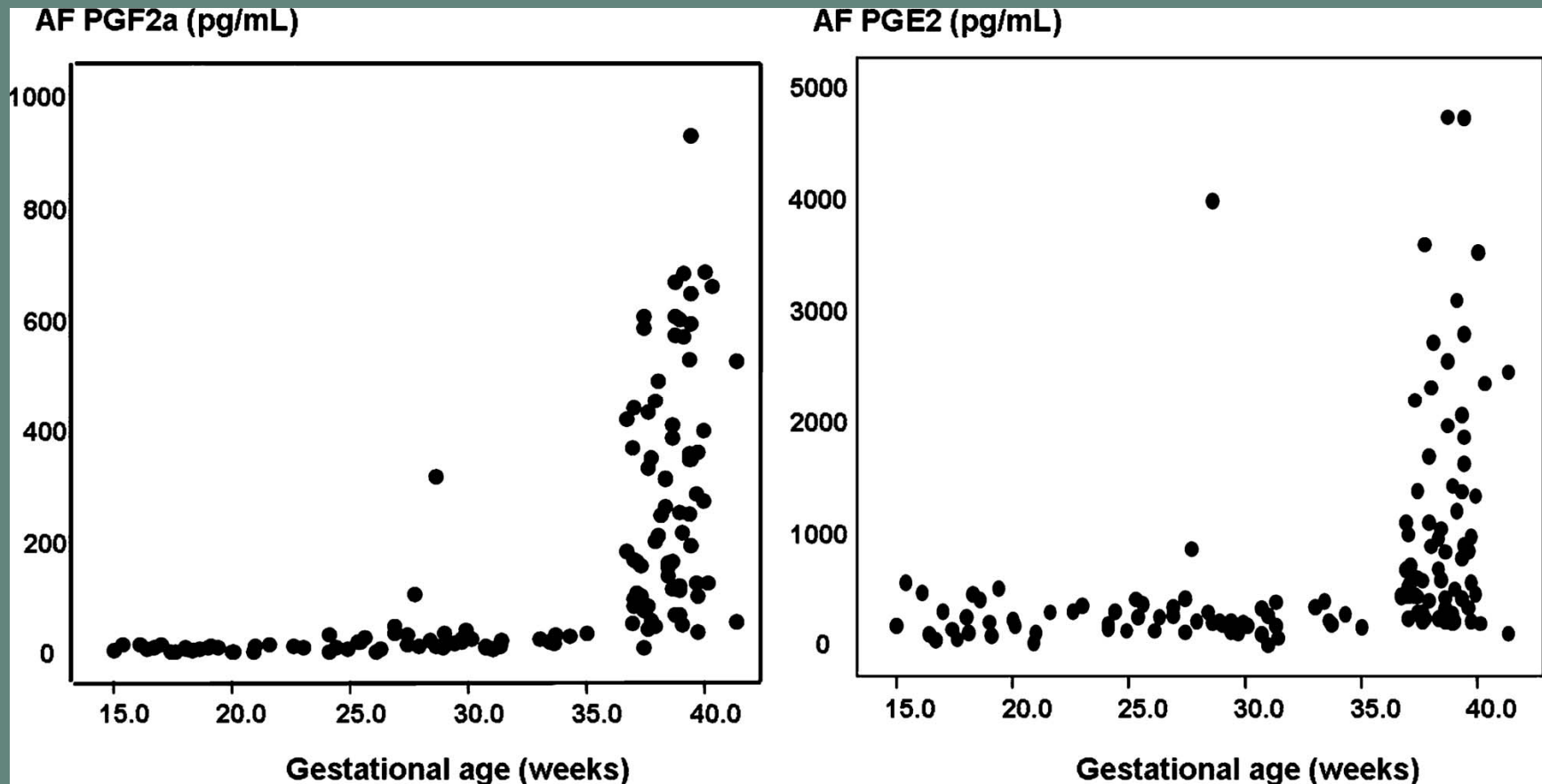
ROS



Increased gestational tissue PTGS2 expression is critical for induction of parturition

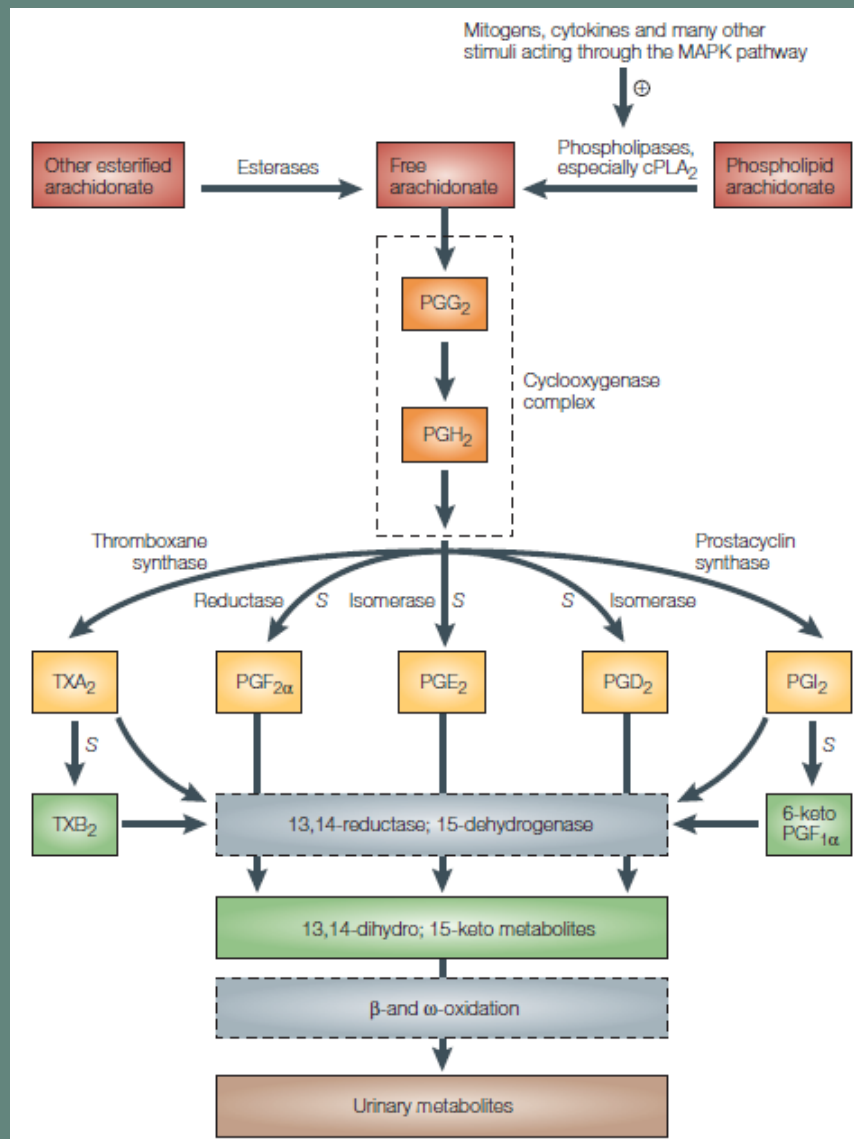


Increased PGF2 α and PGE2 in Amniotic Fluid with Gestational Age



Lee 2008 The Journal of Maternal-Fetal and Neonatal Medicine,

Prostaglandin Screening Assay



Prostaglandin E1

Prostaglandin E2

Prostaglandin F1α

Prostaglandin F2α

Prostaglandin F3α

**Prostaglandin E2
Ethanolamide**

6-keto Prostaglandin F1α

8-iso Prostaglandin F2α

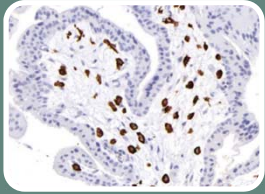
8-iso Prostaglandin E2

Prostaglandin D2

**8-iso-2,3-dinor
Prostaglandin F1α**

Prostaglandin E3

Thromboxane B2

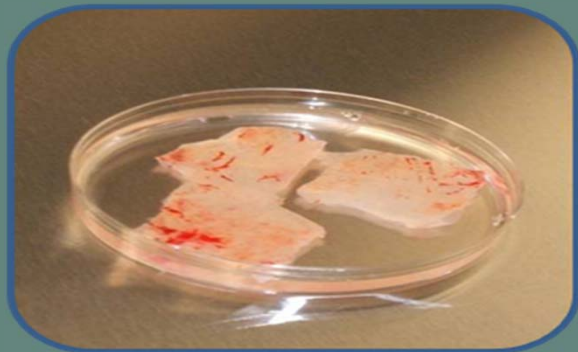


**Primary placental macrophages
secrete increased amounts of total
prostaglandins after a 24-h
exposure to 180 μ M MEHP**

Manuscript under review



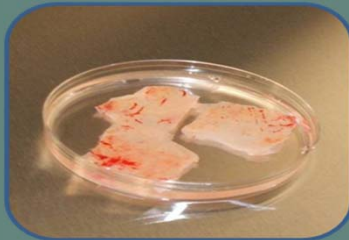
Human Gestational Membrane Explant Model



- Gestational tissue collected from normal, term, not in labor, cesarean deliveries
- 12-mm disks punched from tissue and floated in medium
- Incubated overnight, then treated with MEHP for 24 h



**Explants of Gestational Membranes
secrete increased amounts of total
prostaglandins after a 24- h
exposure to 180 μ M MEHP**



Manuscript under review



Oxidative Stress and Inflammation in US Population

- Used the NHANES databases
 - Publicly available data collected by CDC
 - Exploratory analyses of phthalate metabolite associations with potential response biomarkers
- Putative biomarkers of oxidative stress
 - NHANES 1999-2006 database
 - Bilirubin (a potent antioxidant), inverse association
 - Gamma glutamyltransferase (GGT)

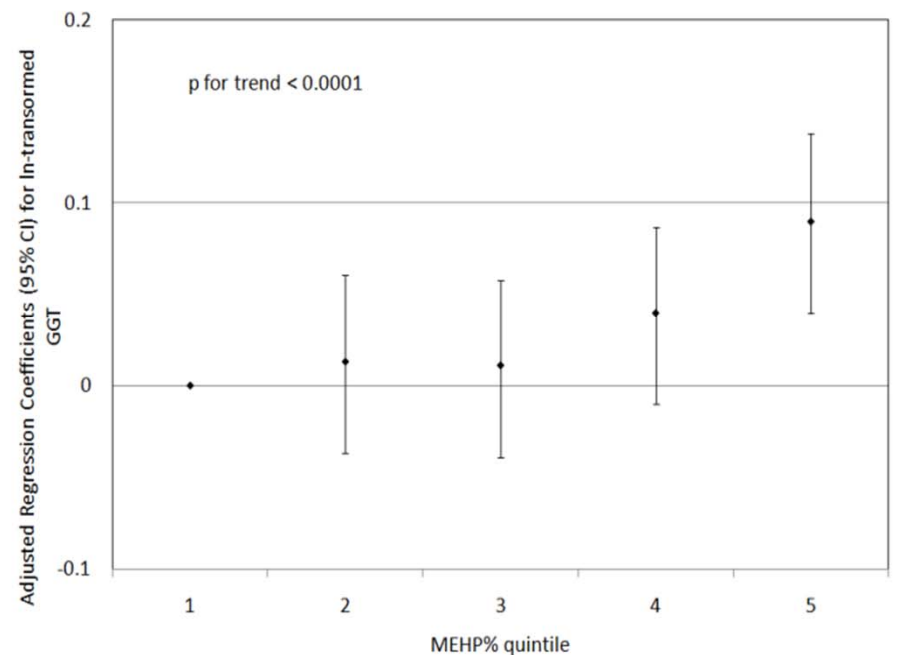
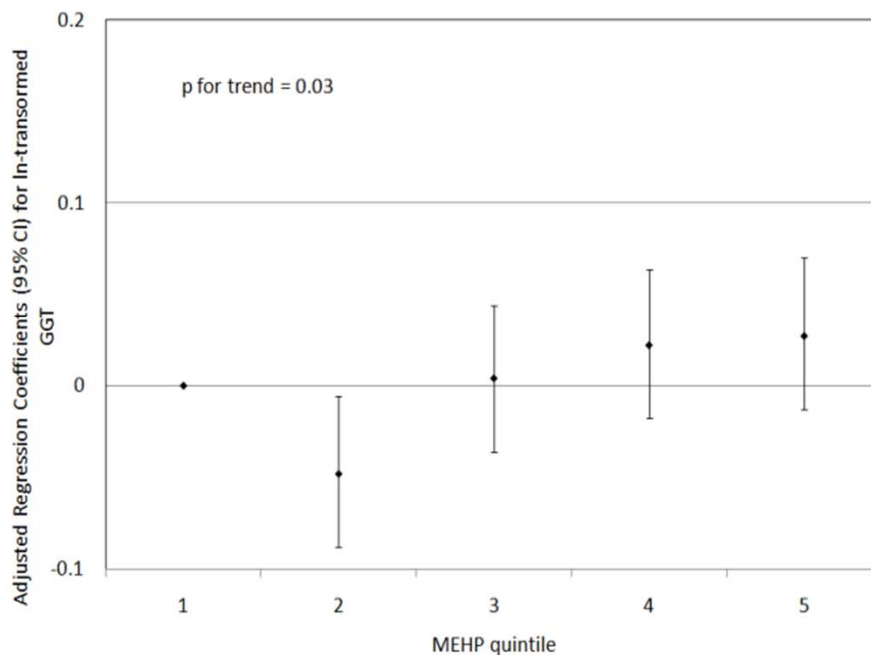


Oxidative Stress and Inflammation

- Putative biomarkers of inflammation (positive associations)
 - NHANES 1999-2006 database
 - C-reactive protein (CRP)
 - Alkaline phosphatase (ALP)
 - Absolute neutrophil count (ANC)
 - Ferritin
 - Fibrinogen



NHANES (1999-2006) Analysis



Adjusted Regression coefficients (95% confidence intervals) for change in GGT associated with urinary phthalate metabolite quintiles. Adjusted for age, sex, race and ethnicity, serum cotinine, income, BMI, and urinary creatinine

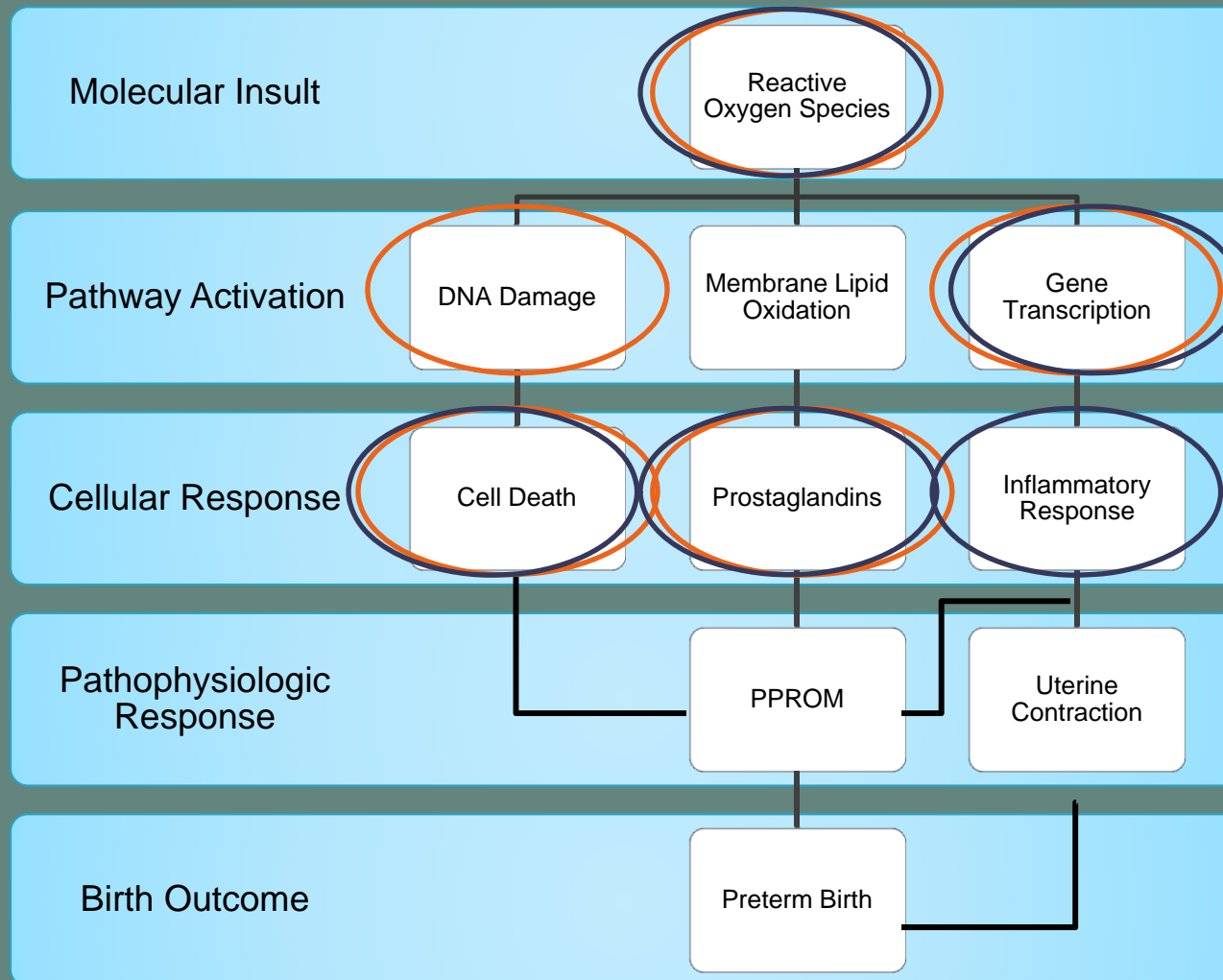
Ferguson KK, Loch-Caruso R, Meeker JD. Urinary phthalate metabolites in relation to biomarkers of inflammation and oxidative stress: NHANES 1999-2006. *Environ Res*; 111(5):718-26, 2011.



Hypothesis Model

MEHP

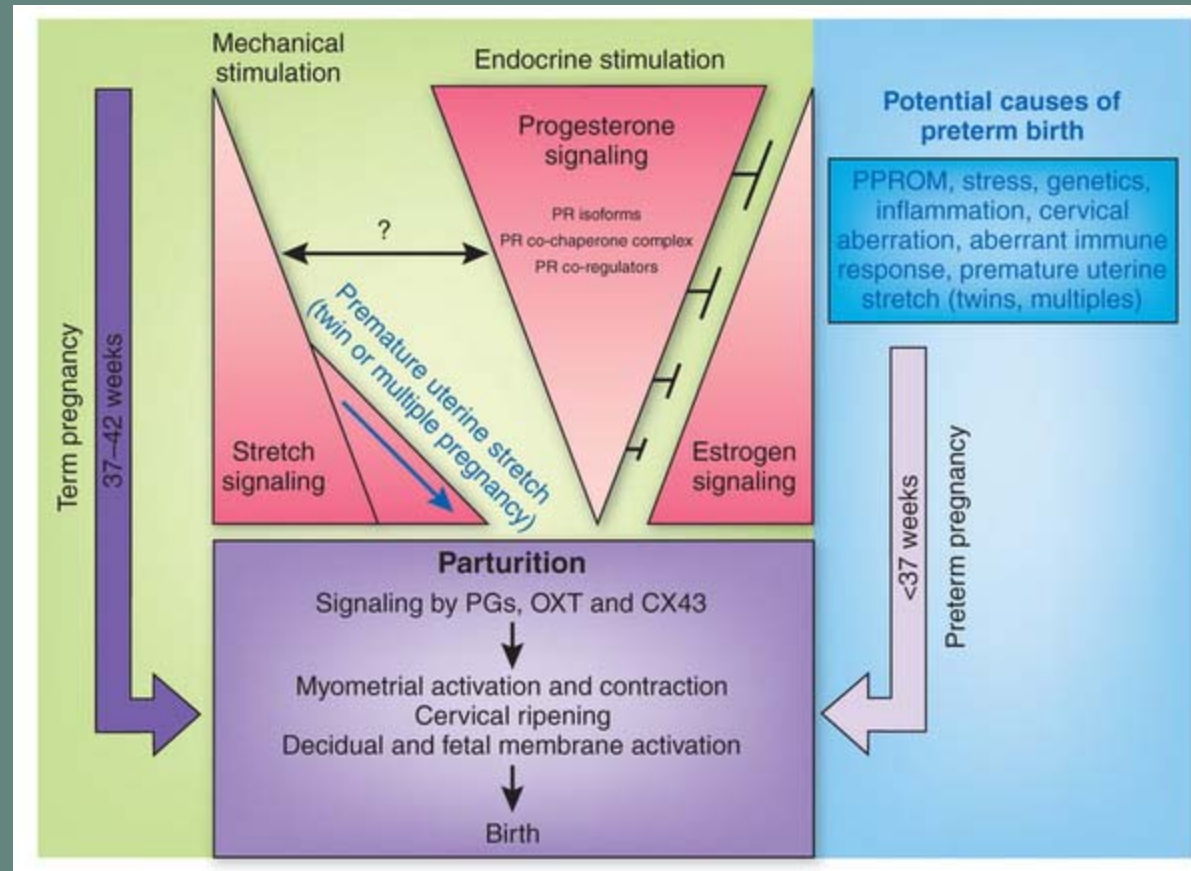
TBHP



PPROM: Premature preterm rupture of membranes



A More Comprehensive Model: The Prematurity Puzzle



Hirota, Y et al. Nature Medicine 16, 529–531 (2010)



Summary



- Phthalates may have a role in preterm birth
- Experimental and human observational data support oxidative stress as a potential mechanistic link
 - May have relevance for other environmental contaminants and human health effects
- Multidisciplinarity is needed to address complex environmental health challenges
 - NIEHS Superfund Research Program Centers facilitate these partnerships

